REMARKS

Reconsideration of the above-identified patent application in view of the amendment above and the remarks below is respectfully requested.

No claims have been canceled or added in this paper. Claims 1, 6-13, 15-20 and 25-28 have been amended in this paper. Therefore, claims 1-29 are pending and are under active consideration.

Claims 1-29 stand rejected under 35 U.S.C. 112, second paragraph, "as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention." In partial support of the rejection, the Patent Office states the following:

> Claims 1-5 and 18-24 are rejected as indefinite because the instantly claimed method lacks a final process step that clearly relates back to the preamble. For example, the method of claim 1, the preamble of the instantly claimed method is drawn to a method for the identification of 5-methylcytosine positions in genomic DNA yet the final process step is that of introducing a detectable label into the heteroduplex; no action of actual identification has occurred. The dependent claims 2-5 and 18-24 also do not have any active step of identifying the desired positions. Method claims require a last step or phrase in the last step that states the accomplishments of the goals for the method, which were stated in the method's preamble. Claim 1 lacks such a last step and is confusing because the additional method step is not sufficiently set forth in the claim or claims 2-5 and 18-24. While minute details are not required in method claims, at least the basic steps must be recited in a positive, active fashion. It is suggested that an amended claim more clearly describing the intended steps be submitted. As such claims 6-17 and 25-27 are also indefinite due to their dependency from claims 1-5 and 18-24.

Applicant respectfully traverses the rejection.

In response to the foregoing ground in support of the rejection, Applicant has amended claim 1 so that an additional step to the method is recited that clearly relates back to the preamble. In view

of the above, the foregoing ground in support of the rejection has been overcome and should be withdrawn.

Also in support of the instant rejection, the Patent Office states the following with respect to claim 1:

> Claim 1 is vague and indefinite due to the lack of clarity in the steps of methodology. The confusing methodology is unclear as to the order in which the method is to be performed in a stepwise manner in addition to what products or intermediates occur and when. The following are some examples: are the two products the nucleic-acid segments; are the nucleic-acid segments the same or two different segments just merely the same in region from which is selected from the genomic DNA (currently the term "the same" would indicate they are but one, however the segment of step b is from another cell therefore contradicting the term "same"); and at what point did the genomic DNA become a segment; is there a difference between the duplex in line 5 versus the heteroduplex of line 10.
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> Applicant is requested to utilize consistent terminology in the claim language; i.e. gene fragments, nucleic-acid segments, segment (alone), fragment (alone); in addition to differentiating between different segments. As such claims 2-27 are also indefinite due to dependency from claim 1.

In response to the above, Applicant has amended claim 1 to make more clear the methodology employed. More specifically, claim 1 has been amended to reflect that the method involves, among other things, carrying out steps a) and b) in an identical manner on two different DNA samples, the products of which are then compared. Step a) must be performed before step b), but step c) may be performed before, after or at the same time as steps a) and b).

In view of the above, the foregoing ground of the rejection has been overcome and should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claim 6:

> Claim 6 recites the limitation that "a 5-methylcytosine was localized in the genomic DNA" in lines 3-4. This lacks antecedent basis because the 5-methylcytosine was not localized, found or identified in any DNA of claim 1 (from which claim 6 depends) or claim 6. As such claims 9, 10 and 27 are also indefinite due to dependency from claim 6.

In response to the above, Applicant has amended claim 6 by replacing the word "localized" with the more correct word "located." Applicant respectfully submits that claim 6, as amended, is definite and that the foregoing ground in support of the rejection should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claims 7 and 8:

> Claims 7 and 8 recite the limitations that cytosine and 5-methylcytosine were found in the genomic DNA of claim 1 as seen for example in claim 7: "pairings occur at the positions at which cytosine was found in the genomic DNA" (lines 2-3). The claims lack antecedent basis because neither the cytosine nor the 5-methylcytosine were found or identified in any DNA of claim 1.
>
> Claim 8 is vague and indefinite due to the lack in clarity as to the further limitation of claim 1 by forming a heteroduplex with a demethylated reference DNA. The mismatch or erroneous base pairing that occurs in claim 8 already occurs in claim 1 in the formation of a heteroduplex with a reference DNA where in the mismatch in the formed heteroduplex indicates the location of a 5-methylcytosine. Thus it is unclear how claim 8 further limits claim 1.

In response to the first point above, Applicant has amended claims 7 and 8 by replacing the word "found" with the more correct word "located." In response to the second point above, Applicant notes that claim 8, as amended, is distinguishable from claim 1 in that, among other

things, claim 8 requires that one of the genomic DNAs be an unmethylated reference DNA and that the erroneous base pairings must be the result of methylation differences between the two genomic DNAs (i.e., not the result of point mutations or other sequence differences between the two genomic nucleic acids).

In view of the above, the foregoing grounds in support of the rejection should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claims 9 and 10:

> The term "sufficiently selective" in claim 9 is a relative term which renders the claim indefinite. The term "sufficiently" as well as "selectively" are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. In addition the phrase itself is confusing in that the terms seem to contradict each other.
>
> The term "sufficiently selectively" in claim 10 is a relative term which renders the claim indefinite. The term "sufficiently" as well as "selectively" are not defined by the claim, the specification does not provide a standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably apprised of the scope of the invention. In addition the phrase itself is confusing in that the terms seem to contradict each other.

In response to the above, Applicant notes that the claims 9 and 10, as amended, no longer recite the language in question. Therefore, the foregoing grounds in support of the rejection are moot and should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claims 11 and 15:

> Claim 11 is vague and indefinite due to the lack of clarity in the claim language "that DNA fragments are obtained in step e) according to claim 1" lines 1-2. The phrase is confusing because step e) of claim 1 merely introduces a label and no DNA fragments are obtained. As such claims 12-17 are also indefinite due to dependency from claim 11.
>
> Claim 15 is vague and indefinite due to the lack of clarity in the claim language "that DNA fragments are obtained in step e) according to claim 1" lines 1-2. The phrase is confusing because step e) of claim 1 merely introduces a label and no DNA fragments are obtained. As such claims 16 and 17 are also indefinite due to dependency from claim 15.

In response to the above, Applicant notes that claims 11 and 15, as amended, no longer recite the language in question. Therefore, the foregoing grounds in support of the rejection are moot and should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claims 16 and 17:

> Claim 16 is vague and indefinite due to the lack of clarity in term "introduced" line 2. It is unclear as to what are the metes and bounds of the parameters that define "introduced" in the context of the "several PCRs of a gene segment." As such claim 17 is also indefinite due to dependency from claim 16.
>
> Claims 16 and 17 are vague and indefinite due to the lack of clarity in the claim language "set stepwise newly each time" (claim 16, line 2) and "positioned newly stepwise" (claim 17, line 2). The language does not make sense thus making it unclear as to what is intended by the Applicant.

In response to the first point above, claim 16 has been amended to replace "introduced" with "carried out." In response to the second point above, claim 16, as amended, no longer recites the language in question. It is respectfully submitted that claim 17, as originally presented, is definite.

In view of the above, the foregoing grounds in support of the rejection should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claim 18:

> Claim 18 is vague and indefinite due to the lack of clarity in the term "chemical function" line 2. It is unclear as to what are the metes and bounds of the parameters that define "chemical function". It is unclear what further limitation has been provided to the primer such as the ability to be further chemical modification, or further modification for binding to another substrate that allows immobilization, or immediate immobilization.

In response to the above, Applicant respectfully submits that one of ordinary skill in the art would understand the metes and bounds of the claim 18. Therefore, the foregoing ground in support of the rejection should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claim 25:

> Claim 25 is vague and indefinite due to the lack of clarity in the term "one" line 1. It is unclear what "one" is referring to.

In response to the above, Applicant notes that claim 25, as amended, no longer recites the term in question. Therefore, the foregoing ground in support of the rejection is moot and should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claim 26:

> Claim 26 is vague and indefinite due to the lack of clarity in the steps of methodology.
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> ...
>
> The claim language does not follow in a stepwise manner that clearly depicts that which Applicant intends for the steps of the method. For example, lines 2-3 (of above) recite the labeling of "the

> immobilized DNA strand" yet nowhere in the claim 26 or claim 1 (from which claim 26 depends) has a strand of DNA been immobilized; in addition it is unclear as to which DNA strand is to be immobilized, the genomic DNA, fragment, segment, etc. The claim continues to recites "the lack of which, after conducting steps d) and e) of claim 1 and a washing step" is confusing as to what is being referred to in "the lack of which". The DNAs within the body of the claim are also confusing as to which is which and at what point: preselected gene segments versus the immobilized DNA strand versus the investigated DNA. Applicant is requested to use consistent terminology to maintain clear and definite claim language.

In response to the above, Applicant has amended claim 26. It is respectfully submitted that claim 26, as amended, is definite. Therefore, the foregoing ground in support of the rejection should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following with respect to claim 27:

> Claim 27 is vague and indefinite due to the lack of clarity of the term "preselection" line 2. The metes and bounds of the parameters required for preselecting a gene segment are unclear.
>
> Claim 27 is vague and indefinite due to the lack of clarity of the term "a more non-specific variant" line 3. It is unclear as to what are the metes and bounds of the parameters that define a "variant", one that is a "non-specific variant", or one that is "more non-specific"; and to what degree is it "more" non-specific.
>
> Regarding claim 27, the word "means" (line 2), it is unclear if the use of the term is an attempt to use a "means" clause to recite a claim element as a means for performing a specified function. If such was intended, then it is necessary for the words which precede "means" to convey a function to be performed. However, currently no function is specified by the word(s) preceding "means," it is impossible to determine the equivalents of the element.

In response to the above, Applicant has amended claim 27 so that the language in question is no longer recited. Therefore, the foregoing ground in support of the rejection should be withdrawn.

Also in connection with the instant rejection, the Patent Office states the following:

> Claim 1 recites the limitations "the identification", "the two products", "the duplex", "the same nucleic-acid segment", "the at-least two amplified products" in lines 1, 5, 6, 8 and 10 respectively.
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> Claim 11 recites the limitation "the cleavage positions" in line 2.
>
> Claim 12 recites the limitation "the analysis" in line 1.
>
> Claim 15 recites the limitation "the fragments" in line 2.
>
> Claim 16 recites the limitation "the primers" in line 2.
>
> Claim 17 recites the limitation "the PCR primers" and "the other primer" in lines 1-2 and 3 respectively.
>
> Claims 18-20 recites the limitation "the PCR" and "the PCR products".
>
> Claim 26 recites the limitations "the mass spectrometer" and "the immobilized DNA strand" in lines 6 and 7.
>
> Claim 27 recites the limitation "the gene segments" in line 2.
>
> Claims 28 recites the limitation "the variable methylation positions" in line 3.
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> As such, claims 2-10, 13, 14, 21-25, and 29 are also indefinite due to dependency from claims 1, 11, 12, 15 and 28.

In response to the above, Applicant notes that claims 1, 11, 12, 15-20 and 26-28 no longer recite the language in question. Therefore, the foregoing grounds in support of the rejection are moot and should be withdrawn.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 1-6, 9-11, 19-25 and 28-29 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over US Patent 5,750,335 (Gifford, May 12, 1998) in view of US Patent 6,017,704 (Herman et al.)." As best understood by Applicant, the Patent Office is apparently contending, with respect to claim 1, (i) that Gifford teaches all of the limitations of claim 1, except for step (a); (ii) that Herman et al. teaches "the process of chemically treating cytosine (without altering 5-methylcytosine) by a bisulfite reaction method in order to identify methylated cytosines (column 6, lines 24-35);" and (iii) that

> [t]hus, it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention was made to perform the genetic mutation detection and identification method of Gifford and further modify treatment of genomic DNA to include chemical [modifications] such as the bisulfite reaction as per the teachings of Herman et al. One of ordinary skill in the art would have been motivated to include chemical modification for the method of identifying methylated cytosines because Herman et al. specifically utilizes the method step for detection of methylated and non-methylated nucleic acids. In addition the required chemical treatment of specific bases of DNA to create non-complementary base pairing is well known in the prior art as stated by Gifford (column 1, lines 38-59) for the purpose of detecting altered base pair behavior.

Applicant respectfully traverses the foregoing ground of the rejection.

The present invention has several advantages as compared to the state of the art. Cytosine methylation plays a very important role in molecular biology and medicine, for example, development, transcription control and cancer. There exists a long-felt need for novel methods for the simple, cost-effective and time-effective detection and analysis of cytosine methylation, both for research and clinical applications.

The present invention makes available such a method. By contrast, Gifford makes no mention of the detection of methylation patterns. Instead, Gifford is limited in its teachings to the detection of hemi-methylated positions by use of specific proteins. This application of the Gifford method is highly restricted to a specific utility and is not suitable for use in a high throughput or research setting.

Moreover, the combination of Gifford and Herman et al. does not render the present invention obvious due to the importance of novel methylation detection technologies and the significant commercial and scientific advantages that result from the applicant's disclosed invention. In particular, the present method enables the identification and localization of differentially methylated positions within a genomic DNA as relative to a reference genomic DNA. This application has particular advantages over the state of the art in basic research as it enables the identification of novel differentially methylated positions in cancer and other diseases. The claimed method presents several improvements over the state of the art at the time in that it represents the first use of the bisulfite modification technique as a tool for the identification of heretofore unknown differentially methylated positions. In general tools for the detection of novel methylated positions such as DMH utilize methylation sensitive restriction enzymes to identify differentially methylated positions throughout the genome. Bisulfite based techniques (e.g., MSP, MS-SNuPE) are best suited for the further investigation of CpG positions which are already known to have variable methylation positions. The development of such assays requires previous knowledge of the sequence context of the CpG position(s) in question and are therefore not suitable for a genome wide survey for novel methylated positions. The only alternative bisulfite based method suitable for the identification of novel differentially methylated positions is sequencing. Sequencing, however, is a costly and labor-

intensive procedure, further compounded by the variable rate of bisulfite conversion, which can render it an error prone technique in non-expert hands.

Gifford enables the identification of genomic mutations whereas Herman et al. enables the sensitive and specific analysis of differentially methylated positions. The method of the present invention has an application distinct from these in that it is a basic research tool. It enables the identification of differentially methylated positions between groups of tissues, thereby enabling the discovery of novel methylation markers, which may provide the basis for further developments in the field of medicine, such as improved cancer detection and disease treatment.

Therefore, the foregoing ground of the rejection should be withdrawn.

With respect to claims 28 and 29, the Patent Office states the following:

> With respect to claims 28 and 29, Gifford does not teach kits that include (un)methylated DNA and corresponding reagents for identification of methylated cytosines.
>
> Herman et al teaches kits for the identification of methylated cytosines that include methylated and unmethylated genomic DNA and reagents necessary for the chemical modifications (bisulfite reaction) of the DNA (column 18 line 60 - column 19, line 15).
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> Thus, it would have been prima facie obvious to one of ordinary skill in the art at the time of the invention was made to perform the genetic mutation detection and identification method of Gifford and further modify the kits for performing methylation detection to include the reagents for DNA chemical treatment and methylation as per the teachings of Herman et al. One of ordinary skill in the art would have been motivated [to] modify the kits of Gifford (column 6, lines 51-64) to include reagents for methylation and (un)methylated DNA as required by claims 28 and 29 for completion of the described method for methylation mutation identification as suggested by Gifford (column 28, lines 64-66).

Applicant respectfully traverses the foregoing ground in support of the rejection. Applicant respectfully submits that, for at least the reasons given above, there is no basis for combining the references in the manner proposed by the Patent Office.

Therefore, the foregoing ground of the rejection should be withdrawn

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claims 12-17 stand rejected under 35 U.S.C. 103(a) "as being unpatentable over US Patent 5,750,335 (Gifford, May 12, 1998) in view of US Patent 6,017,704 (Herman et al.) as applied to claims 1-3, 5, 6, 9-11, 19-25, 28 and 29 above; and further in view of WO 97/33000 (Monforte et al., 12-Sept-1997)."

Applicant respectfully traverses the foregoing rejection. Claims 12-17 all depend ultimately from claim 1. Claim 1 is patentable over Gifford in view of Herman et al. for at least the reasons discussed above. Monforte et al. fails to cure all of the deficiencies of the combination of Gifford and Herman et al. Therefore, based at least on their respective dependencies from claim 1, claims 12-17 are patentable over the subject combination of Gifford, Herman et al. and Monforte et al.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claim 8 stands objected to under 37 CFR 1.75(c) "as being of improper dependent form for failing to further limit the subject matter of claim 1."

Applicant respectfully traverses the foregoing objection. As noted above, claim 8 is narrower than claim 1 since claim 8 recites further limitations, such as that at least one of the genomic DNAs is an unmethylated reference DNA. Accordingly, for at least the above reason, Applicant respectfully submits that the foregoing objection should be withdrawn.

Claim 13 stands objected to "due to the improper abbreviation applied to a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Currently the claim recites 'MALDI' whereas the appropriate abbreviation is 'MALDI-TOF'."

In response to the above objection, Applicant has amended claim 13 as suggested by the Patent Office. Accordingly, the objection has been overcome and should be withdrawn.

Claim 27 stands objected to "because of the following informalities: the instant claim is improperly dependent from claims 1-25; claim 27 must claim dependency from claims 1-25 in the alternative, for example 'any one of claims 1-25'."

In response to the foregoing objection, Applicant notes that claim 27 has been re-written in independent form. Accordingly, the foregoing objection is moot and should be withdrawn.

The disclosure stands objected to because the specification does not employ "the preferred layout for the specification of a utility application."

In response to the foregoing objection, Applicant notes that the use of the preferred layout referred to by the Patent Office is not mandatory. Nevertheless, Applicant has amended the present specification to include certain section headings that bring the disclosure more in line with the preferred layout. As a result, Applicant respectfully submits that the foregoing objection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is now in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

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I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Mail Stop Fee Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on April 5, 2004.

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